2. Although each substance has its particular advantages and indications as a test, indigocarmin, at least for the purposes of the surgeon, especially in the diagnosis and prognosis of unilateral renal disease, seems just as useful and possibly more practical than the new drug phenolsulphonephthalein.

3. Phenolsulphonephthalein in many respects is an ideal substance for employment in studying the pathology and physiology of the kidney. It may possibly be more sensitive than indigocarmin, in fact, may prove to be too delicate. On the other hand, the technique of the test is extremely simple and may be employed painlessly. Preference should be extended to this drug over indigocarmin whenever it is desirable to learn the total or combined efficiency of both kidneys.

In conclusion, I desire to express my grateful appreciation to Drs. Edsall, Frazier, Riesman, and T. T. Thomas for permitting me to use material that made it possible to conduct, in part, these studies.

ACTINOMYCOSIS IN NORWAY: STUDIES IN THE ETIOLOGY, MODES OF INFECTION, AND TREATMENT.

By Frances Harbitz,
PROFESSOR OF PATHOLOGICAL ANATOMY AND GENERAL PATHOLOGY AT THE UNIVERSITY OF CHRISTIANA,
AND
Nils B. Grondahl,
FIRST ASSISTANT AT THE INSTITUTE FOR PATHOLOGICAL ANATOMY.

Actinomycosis is far from being a rare disease. Cases have now been studied in nearly every country. In America, Hodenpyl[1] published studies on this disease in 1890, and 1899 Ruhräh[2] reported a large series of cases observed in America.

The first case of actinomycosis was reported in Norway as early as 1887, and since that time scattered cases have occurred, but up to this time a collective report has not been brought forth. During the last fifteen to twenty years the Institute for Pathological Anatomy has been investigating a large series of instances of actinomycosis. The material was obtained partly from autopsy and partly from pathological material sent to us by the practicing physicians in various parts of Norway.

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[1] Hodenpyl, 1890, Actinomycosis of the Lungs, N. Y. Med. Record, 1890, and Erving, W., Actinomycosis Hominis in America, with Report of Six Cases. Johns Hopkins Hospital Bull., 1892.

[2] Actinomycosis in Man, Annals of Surgery, October to December, 1891.

Etiology. Our series includes 87 cases, and the lesions were distributed as follows: 45 per cent. occurred on the neck and face (all cured); 23 per cent. in the thorax (all dead); 30 per cent. in the abdomen (19 dead, 5 cured); 3 per cent. on the skin (1 dead, 1 cured).

We have reasons to believe that this represents approximately the relative frequency on various parts of the body: 56 occurred in men, 27 in women, and in 7 the sex was not given. The relative age is given: 2 occurred in the first decade (boys); 12 occurred in the second decade (10 men, 2 women); 26 occurred in the third decade (17 men, 9 women); 16 occurred in the fourth decade (10 men, 6 women); 13 occurred in the fifth decade (8 men, 5 women); 7 occurred in the sixth decade (4 men, 3 women); 3 occurred in the eighth decade (3 men).

Among the 39 cervico-facial cases, in 15 the lesions were located on the cheek especially on the maxilla, the forehead, and the parotid region; in 20 they were on the mandibular or on the submental region and in 3 they were located farther down the neck, and one tumor was on the tongue.

The infection atrium was in every instance the mucous membrane of the oral cavity, and often the part covering the gums. There was no evidence of the fungi having entered by carious teeth as is supposed to take place by Lord.

All the 39 cases recovered.

In our thoracic group the primary lesion was always in the lungs, and, as a rule, in the lower lobes. The disease simulated an atypical bronchopneumonia or pulmonary tuberculosis, with fever, dyspnea, cough, and a rusty tenacious sputum, which, as a rule, contained the characteristic sulphur granules. Everyone of these terminated fatally.

The average duration was one to one and one-half years. One lived on for eight years.

The primary lesion of the abdominal cases was some part of the alimentary tract, most often the vermiform appendix. There were no primary lesions in the rectum, bladder, or genital organs, although gravitation abscesses did extend into these organs. The disease occurred as localized abscesses most often in tne ileo-cecal region. These spread both extra- and intra-peritoneally. Liver abscesses were frequently found. The average duration of the fatal cases was one to two years.

In the two patients where the disease was primary in the skin, one had a localized abscess on the arm which soon healed. The other had a phlegmonous infection of the leg, that originated from an ulcer that started during the harvest. This man's leg was amputated, but he died.

Pathological Anatomy. The lesions occur in the form of a chronic suppurative inflammation with marked infiltration and a relatively small amount of a thick tenacious pus. The characteristic feature of actinomycotic inflammation is its advance through all tissues or organs with the development of fistulous tracts. These lead into abscesses containing the typical pus. The internal organs are attacked from the periphery, and large abscesses always result. In the later stages general infection occurs through the blood with the formation of abscesses in the lungs, liver, kidneys, etc. It does not spread through the lymphatics except near the very end of the disease. In this way actinomycosis differs from other chronic inflammations, such as tuberculosis or glanders. Microscopically nothing striking is seen. Marked hemorrhagic infiltration is often observed and the giant cells are abundant.

The striking and perhaps pathognomic thing in actinomycotic inflammation is the presence of the sulphur granules. These are formed from mycelia and often contain the so-called clubs. They vary in size, color, and consistency, and there are rather marked differences between those found in man and cattle. In man the granules are usually pin-head sized; they are round or oval; of a grayish-white or yellowish color and usually of a soft consistency; rarely are they infiltrated with calcium. The color may be green or even brownish-black. With low magnification they appear as light yellow rosettes, consisting of a central mass of mycelia which radiate toward the periphery forming radial stripes (Fig. 1). The granules are surrounded by a dense zone of leukocytes. With the high power one may at times notice club formation at the ends. If they are present, the virulence of the fungus is low. Soft small granules are always found in rapidly growing lesions. In actinomycosis bovis the granules are larger, more distinct, and have a decided yellow color. They show club formation and are often partially calcified (Figs. 2, 3, and 4). They consist then of degenerated threads having very scant power of growth on media. Spores have never been seen.

Remembering then that the characteristic things in actinomycosis are a chronic inflammation advancing through all tissues, producing fistulous tracts from which ooze a thick tenacious pus containing sulphur granules and that generalized infection occurs only through the blood stream we pass to a study of the cultural characteristics of the fungus.

Cultural Characteristics. Much work has been done by many investigators in different countries for the purpose of determining the typical growth of the pathogenic fungus or fungi, and greatly varying results have been obtained; thus Boström and a large number of others have isolated a fungus growing in aërobic cultures producing aerial hyphæ, and showing true branching; it also colors the media yellow. This type has been identified with the actinomyces occurring in nature. Then another group of workers among which are Wolff, Israel, J. H. Wright,[3] and Shiata,[4] have isolated an anaërobic fungus from actinomycotic lesions in men. Besides these two, a number of streptothrix more or less similar to the above have been cultivated from chronic inflammations where no sulphur granules occurred.

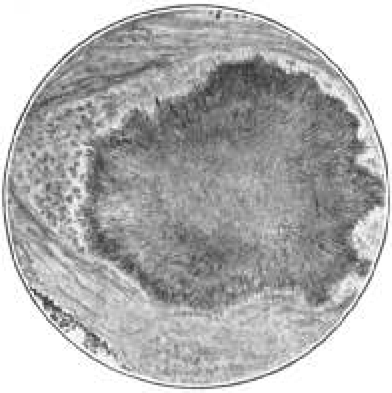

FIG. 1.—Human actinomycosis. Distinct radiation but indistinct bulbs. × 300.

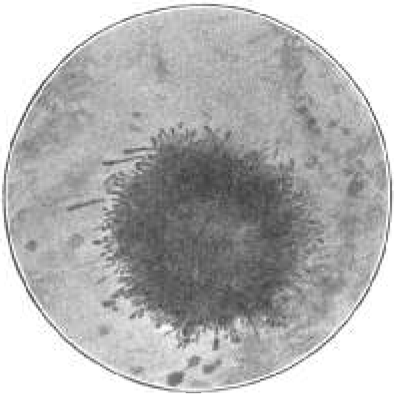

FIG. 2.—Bovine actinomycosis. Granules with marked calcification. × 300.

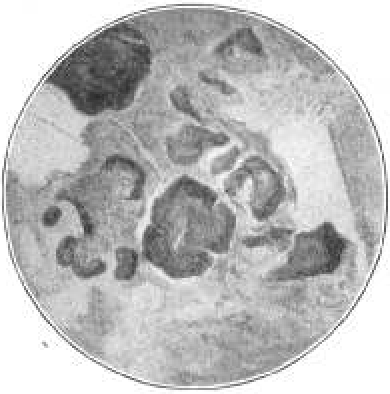

FIG. 3.—Bovine actinomycosis. × 80.

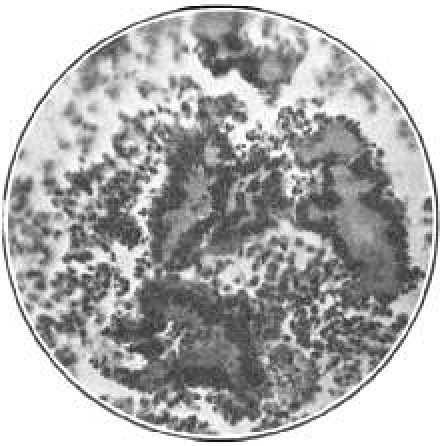

FIG. 4.—Bovine actinomycosis. Granules with marked calcification in the periphery; centre homogeneous without definite thread formation.

Because of this uncertainty as to which type was the real cause of the actinomycotic inflammation we decided to carry on a series of experiments to determine the exciting factors in our cases, and a brief statement of our work follows.

During the last year we made cultures of all the cases secured, but obtained pure cultures from only about half of them. Growth was more easily obtained from the small soft grayish granules. Pure cultures were obtained from 10 cases and seventeen strains were isolated, partly by making cultures at different times from the same lesion, and partly by inoculating media from pus, urine, or sputum from the same patient. If we add to our results the results of investigations carried on at the Pathological Institute where pure cultures were obtained from 5 cases by Dr. Kjerschow and 5 by Professor Harbitz, it makes a total of 20 cases with twenty-seven strains. Eleven of these were from the cervical facial types, three from the lungs, and four from the intestines.

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[3] Biology of Microörganism of Actinomycosis, Pub. of the Mass. Gen. Hosp., 1905, vol. i, No. 1.

Beiträg zur Kenntniss die menschlichen Actinomycosis, Deut. Ztschr. f. Chir., 1909, Band ci.

METHOD. Pure cultures were obtained by putting the granules on slant agar and leaving them in daylight and at room temperature for three days. Then subcultures were made on the ordinary aërobic and anaërobic media and the cultures kept at 37° C. By this method we grew an anaërobic type, agreeing in all essentials with the one isolated by J. H. Wright. In agar stabs, the anaërobic growth was visible on the third day, and by the ninth colonies the size of a pinhead appeared as a dense zone about one centimeter from the surface. Each colony was similar in structure to a sulphur granule. Practically no growth appeared on aërobic agar slants, but it grew luxuriantly under anaërobic condition. Aerial hyphæ were never observed. In bouillon cultures grew aërobically but especially well anaërobically. Milk was not coagulated. Growth did not occur on glycerin serum, potato, gelatine, peptone broth, or raw eggs.

In order to keep the cultures alive it was necessary to transplant them about every tenth day. However, we did obtain a culture from a one hundred and forty-five day old granule. No odor, gas, or color was produced. The fungus grew very well in agar to which 1 to 2 per cent. of potassium iodide had been added. The fungus showed absolutely no tendency toward adopting an aërobic habitat, though we kept one strain through twenty generations. In old cultures the threads were branched and difficult to stain.

With the anaërobic fungus thus obtained we now carried out animal inoculations; the animals used were rabbits and guinea-pigs. Inoculations were made intraperitoneally, intrapleurally, and subcutaneously, first with sulphur granules and only once did an abscess develop in the pus of which were found granules, but this was probably not due to a real growth of the fungi. Inoculations from cultures, too, were negative. We also grew a pure culture of an anaërobic fungus, from an actinomycotic abscess in a cow, and made animal inoculations, but again failed to obtain results.

From the above we conclude that the anaërobic fungus isolated by us from many cases of actinomycosis hominis is the exciting cause of the disease in man, in at least the large majority of cases, and this conclusion is greatly strengthened by the fact that our results are practically identical with the ones obtained by Wolff, Israel, J. H. Wright, and Shiata. It is also probable that the organism producing the disease in cattle is identical with that in man, but further work must be carried on to substantiate this.

We also examined a number of other strains supposed to be the cause of actinomycosis, but they were all aërobic and differing widely from ours. Having thus determined that the anaërobic fungus was the true cause of actinomycosis, it was left to find out, if possible, just what relation it has to the actinomyces found in nature; for botanists have obtained pure cultures and thoroughly studied many different varieties. Since we lacked time to carry on experiments to determine this relation we persuaded an able botanist, Mr. Hagen, to investigate this matter. Mr. Hagen obtained pure cultures of three strains from the air, but they were all aërobic fungi, and so were the two pure strains he secured from straw. He also grew three pure cultures from barley and oats; everyone of these had marked aërobic tendencies as had also a pure culture from manure. It might be added here that Mr. Hagen made an inoculation from an actinomycotic lesion and recovered a pure culture of the anaërobic fungus we had isolated. From his work, Mr. Hagen concluded that actinomyces hominis occurs rarely if at all in nature and if it does occur, it must exist under certain conditions only. His experiments, however, are too few from which to draw definite conclusions.

If the pathogenic fungus does not occur in nature, how, then, is the disease transmitted? Here it must be admitted that the mode of transmission is by no means clear. We have already shown that the fungus gains foothold through the lungs, the mucous membrane of the mouth or that of the intestines, but whence does it come? Is it found in the organism or on the outside and through what medium does it enter tissues? These are questions yet to be solved.

Up to this time it has been taken for granted that the fungus producing the disease in men is identical with the exciting cause of actinomycosis bovis. This has not been definitely proved. Actinomycosis hominis has also been thought to occur more frequently among country people associated with cattle. This we have found not to be the case. It is as common in cities as among farmers. Infection to man from cattle is, therefore, not probable, and since the disease always occurs as isolated cases in far separated localities, we are not justified in assuming that direct infection from man to man occurs.

It has been supposed that the pathogenic fungi are carried to the tissues by straw, slivers, and other parts of plants. This hypothesis has been based on two things: (1) That the natural and the pathogenic fungi are identical. This we have shown to be erroneous. (2) That straws, slivers, etc., have often been found in actinomycotic abscesses. Pieces of plants are found especially frequently in the mouth lesions of cattle (Fig. 5). One must bear in mind, however, that these might have entered the abscesses secondarily. But straws have also been found in cervico-facial abscesses in man. To be sure, such findings are much rarer in man than in cattle, never-

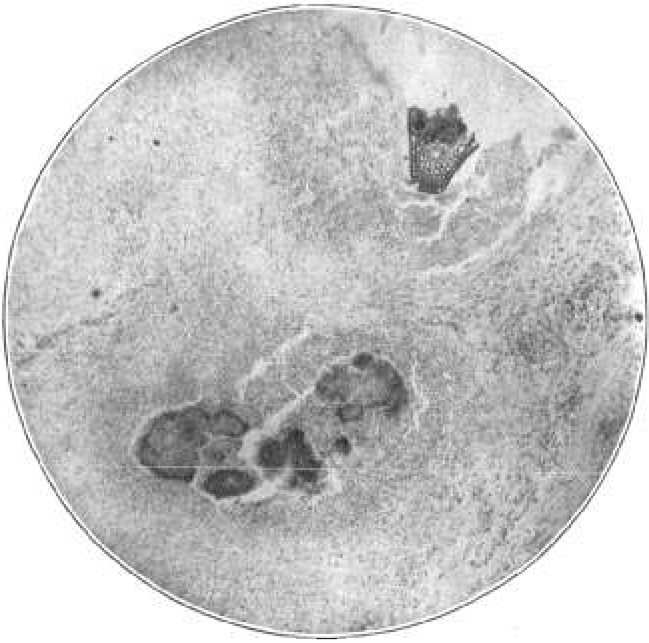

FIG. 5.—Actinomycelial granule lying beside an awn in actinomycotic bovine granulation tissue. × 80.

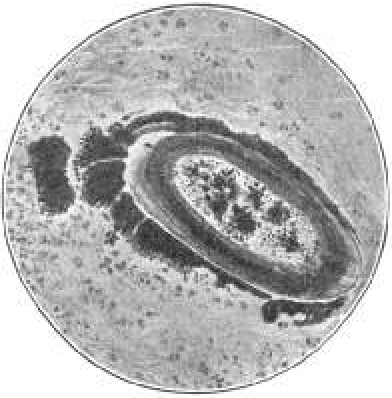

FIG. 6.—Cross-section of awn with actinomycosis in the central canal and growing outward. From bovine tongue. × 300.

theless, pieces of plants do occur. It is of special interest to note that abscesses always developed after a latent period varying from months to years after the entrance of such *corpora aliena*. Abscesses have also occurred after puncture of the mucous membrane with wooden splints, fishbones, pieces of glass and in one of our patients, after an abrasion on the leg. The finding of mycelial threads in or around these objects has served to strengthen the idea that they are the real carriers of the infection (Fig. 6). There is then some definite evidence showing that straws, splinters, etc., do play an indirect role in the transmission of actinomycosis, but there is certainly nothing to prove that the disease is directly transmitted by them.

Since the fungus is not carried directly from the outer world into the animal tissues the idea that it lives as a saprophyte on the mucous membrane and enters the tissue to produce lesions naturally suggested itself. It must also be remembered that infection does not take place by the granules, since they are a pathological product produced after the fungus has gained foothold. Infection then must occur either through threads or by some unknown form. Two possibilities confront us—(1) either the fungus lives as a saprophyte on plants to enter man by chance and then to go on living a saprophytic existence until a *locus minoris resistentiæ* is offered, when it becomes at once pathogenic, or (2), as J. H. Wright has suggested, the fungus is pathogenic from the start and lives on the mucous membrane ready to enter the tissues when aided by foreign bodies. Where the fungus might originate, Wright does not suggest. Both the above hypotheses depend on the demonstration of the fungus on the mucous membrane. What is known concerning this?

Mycelia, thought to be actinomycotic, have been found by Miodowski in the tonsils and the ductus lacrimalis. But much weight can not be placed on these findings since cultures were never made.

But we obtained from the tear sac of a student who had been suffering for over a year from a chronic dacryocystitis, a granule consisting of mycelia, and closely simulating the sulphur granules of actinomycosis. From this granule inoculations were made and a pure culture of an anaërobic fungus was grown. The cultural characteristics of this strain were very much like the ones of the fungus grown from actinomycotic lesions. Whether this fungus was the cause of the dacryocystitis or not is hard to determine, but the finding of it does indicate that the fungi may be present on the mucous membrane.

Finally, a word about the existence of actinomyces in carious teeth. Professor Partsch[5] and his pupils have reported the findings of numerous sulphur granules in decayed teeth and in the pus of perialveolar abscesses. These again simulated those producing actinomycosis only morphologically. No cultures were made. J. H. Wright and Lord[6] carried on some very interesting experiments along this line. They obtained from carious teeth mycelial threads like those of the pathogenic fungus. These they inoculated into guinea-pigs and observed the formation of abscesses containing typical sulphur granules. Unfortunately they did not recover the fungus in pure cultures. We also inoculated material from carious teeth taken both from people who were well and from patients suffering from actinomycosis, but our results were invariably negative.

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[5] Jaehn, Die Acktinomycosis des Mundes, Deut. Monatschr. f. Zahnheilk., Heft 1 to 3, 1909.

It is plain from the above that nothing very definite is known about the habitat of the actinomyces hominis prior to its attack on the tissues. Further investigations along this line are called for.

TREATMENT. In conclusion, a few words about the treatment of this disease. Up to the present time it has consisted of local surgical treatment and internal medication. Locally the lesions is removed entirely when practicable, but this can seldom be done, hence surgeons often resort to curettage or simple incision. Local injections of potassium iodide have been tried. Potassium iodide has also been given internally and good results claimed. But since we have shown that the fungus grows luxuriantly in 1 to 2 per cent. potassium iodide, its curative value is doubtful. The behavior of the disease is so extremely varied that the effect of any treatment is hard to judge.

The fact that a great variety of medicines have been recommended in the treatment of actinomycosis plainly shows the need of a specific treatment. But, in order to obtain any kind of serum therapy, great obstacles must be overcome: (1) The organism is hard to grow, their pathogenicity for lower animals is slight, to say the least. (2) The production of toxins is scant. (3) It is next to impossible to use the mycelia in agglutination tests.

French investigators have developed a serum which will coagulate spores of streptothrix. This serum is secured from various mycotic diseases, including blastomycosis, but this, even at its best, can only be used in differentiating mycotic diseases from those of bacterial origin. We tried their methods, but obtained only negative results. We also tried the fixation of the complement according to Wassermann's technique, but the results were not satisfactory. Hence we conclude that agglutination tests and fixation of the complement are of no clinical value in the diagnosis of actinomycosis.

Since practically no work has been done so far on a specific vaccine,[7] we undertook to see what could be done in this line. Our method was briefly as follows: It is impossible to count the microbes; we took, therefore, three pea-sized bouillon cultures, ground them up in 5 c.c. of normal salt solution and put them in a shaker for twenty-four hours. The mixture was then drawn up into long glass tubes, heated to 80° for three hours, and using this as a stock mixture we made two solutions from it: (*a*) 1 c.c. of the mixture plus 50 c.c. of 0.9 per cent. NaCl plus 2 drops of lysol: (*b*) 4 c.c. of the mixture plus 50 c.c. of 0.9 per cent. NaCl plus 2 drops of lysol. From solution *a*, $\frac{1}{4}$ to 1 c.c. was injected subcutaneously every second and third day, later solution *b* was tried, using 1 c.c. every second day. We never observed any local reaction from these injections. With this vaccine two patients suffering from a severe infection were treated, with the following results.

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[6] A Contribution to the Etiology of Actinomycosis, Boston Med. and Surg. Jour., July 21, 1910.

[7] J. H. Wright has told us personally he has tried two cases, Dr. Jensen has used it, and Wynn reported a case successfully treated with a sort of a vaccine.

The first patient was a boy suffering from abdominal actinomycosis and with several large abscesses in the liver. He received five doses of solution *a* at intervals of from two to four days. The injections had apparently no effect, and the case went on to a fatal termination. The second, a man, aged twenty-two years, also suffered from the abdominal type. He had several large abscesses and one had penetrated the bladder. For three months this man received every second day an injection, in the beginning of solution *a* and later of solution *b*. At first he seemed to improve but soon his fever increased, dyspnea appeared and with it cough and pain in the epigastrium. After a few weeks, however, he began to improve and from then on the improvement was steady and marked. Today he is working and is practically cured, but there yet remains a soft swelling on the left side.

We have then one good result, but of course, no great weight as to the value of the treatment can be placed on it. Further trials with a specific therapy are certainly justifiable.

CONCERNING THE PRESENCE OF THE EMBRYOS OF TRICHINELLA SPIRALIS IN THE BLOOD OF PATIENTS SUFFERING FROM TRICHINIASIS.

By Albert R. Lamb, M.D.,

EXTERNE PATHOLOGIST, PRESBYTERIAN HOSPITAL, NEW YORK.

(From the Second Medical Division, Presbyterian Hospital.)

It is now two years since Herrick and Janeway[1] first demonstrated the embryos of Trichinella spiralis in the blood of a patient suffering

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[1] Demonstration of the Trichinella Spiralis in the Circulating Blood in Man, Archives of Int. Med., 1909, iii, 263.